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Original Article

FORMULATION AND EVALUATION OF LIPOSOMES CONTAINING SORAFENIB TOSYLATE

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ABSTRACT

The aim of the present study is to encapsulate Sorafenib tosylate in liposomal formulation for the effective treatment of hepatocellular carcinoma. Many conventional dosage forms of Sorafenib tosylate are available in market with high drug dose owing to high permeability and low solubility. But the large drug doses are coupled with a number of toxicities. To overcome such problems, the liposomal inclusions of Sorafenib tosylate have approached with the objective of increasing its bioavailability with small drug dose and better tumor targeting by making as a nano-sized formulation. The compatibility study of drug with phospholipids & other excipient have checked using the FTIR technique. In the present study, Sorafenib tosylate liposomes have prepared by thin film hydration technique using soyalecithin as lipid coat, cholesterol as rigidator, tween 80 and organic solvent like chloroform and methanol with hydrating media phosphate buffer (pH 7.4). Six formulations of liposomes have formulated, characterized and evaluated by particle size of vesicles, zeta potential, surface morphology, entrapment efficiency, invitro drug release and stability studies. The optimized formulation containing drug, lipid and cholesterol ratio 1:8:3 respectively, showed highest entrapment efficiency (55.62%). The optimized formulation has exhibited 83.36 % drug release within 24 hours. The stability study as per ICH guidelines at different temperatures conducted has showed maximum liposomal drug retention at refrigerated temperature 4ºC as compared to room temperature and accelerated stability study. The results suggest that the liposome encapsulation can increase the bioavailability of highly potent poorly bioavailable Sorafenib tosylate and can be used as a useful targeted drug delivery system for an effective management of hepatocellular carcinoma.

KEY WORDS: Sorafenib Tosylate, Film Hydration Method, Soya Lecithin, Cholesterol, In-Vitro Drug Release & Stability Studies

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INTRODUCTION

The term Liposomes were first coined by Bangham in 1965, the name liposome was made of two Greek words first “lipos” means fat and “somas” means body. Liposomes are microscopic vesicles composed of one or more lipid bilayers which have the spherical shape and size of liposome ranging from 20 to 1000 nm. Drug molecules can either be encapsulated in the aqueous space or entrapped into the lipid bilayers. The exact location of a drug in the liposome will depend upon its physicochemical characteristics and the composition of the lipids [1, 2, 3, and 4].

Liposomes are colloidal dispersion formed as concentric biomolecular lipid vesicles that are capable of encapsulating drugs. These lipid vesicles are usually of phospholipids with or without some additives. Cholesterol has to be added to improve bilayer characteristics of liposomal cells like membrane rigidity of the artificial vesicles [5, 6, 7, 8].

The liposomes can penetrate the drug to target cells for example; cancerous cell more selectively and decrease the possible side effects of usual chemotherapy like nausea, hair loss and vomiting. Sorafenib inhibits tumor cell proliferation and vascularization by the activation of the receptor for tyrosine kinase signaling in the Ras/Raf/Mek/Erk cascade pathway. Sorafenib is an effective chemotherapeutic agent against the various tumor types and inhibits proliferation, angiogenesis, and invasion of tumor cells. However, poor bioavailbility of sorafenib limits the clinical application for treatment of hepatocellular carcinoma. That hindrance might be overcome by use of liposomes for tumor specific drug delivery and controlled release of sorafenib [9, 10, 11, and 12].

2. MATERIALS & METHODS

2.1 Materials

Sorafenib tosylate obtained as a gift sample from Cipla Pvt. Ltd. Vikhroli West, Mumbai, India. Gift sample of Soyalecithin from Lipoid GMBH, Frigenstrasse 4, Ludwigshafen, Germany. Cholesterol was purchased from Research Lab Fine Chem Industries, Mumbai. The other chemicals, reagents and solvents used like potassium chloride, sodium chloride, potassium dihydrogen phosphate, disodium hydrogen phosphate, chloroform, and methanol were of analytical reagent grade.

2.2 Pre-Formulation Studies

2.2.1 General Procedure for the Preparation of Calibration Curve by UV

A standard stock solution of pure drug 1 mg/ml concentration has been prepared with methanol. Then; 2, 4, 6, 8, 10 and 12 ml stock solution have withdrawn and diluted with phosphate buffer (pH 7.4) to make 0.2, 0.4, 0.6, 0.8, 1 and 1.2 µg/ml concentration respectively in a series of 10 ml volumetric flasks. The absorbances of these solutions have taken at 265 nm, using 1 cm quartz cuvette in UV- Visible spectrophotometer. Absorbance values were plotted against respective concentration to obtain standard calibration curve [13].

2.2.2Drug-Excipient Compatibility Studies

The compatibility between the drug, chosen lipids and other excipients has been checked by using FTIR peak matching method. FTIR study has no appearance or disappearance of peaks in the drug-lipid mixture, which confirmed the absence of any chemical interaction between the drug, lipid and other chemicals. The drug excipient compatability studies was studied by comparing the interpretation data of FTIR of pure drug with the FTIR of various excipients like phospholipids, cholesterol & finally with liposomal formulation [14].

2.3 Procedure for the Preparation of Sorafenib Liposome

The six formulations of liposomes (Table 1) were prepared by dried thin film hydration technique using a rotary evaporator. The drug soyalecithin and cholesterol were dissolved in 5 ml of chloroform (3.5 ml) and methanol (1.5 ml) mixture in 250 ml round bottom flask. The organic phase was evaporated under vacuum using rotary flash evaporator, which allows soyalecithin to form a thin dry film on the walls of the flask. This system was maintained at vacuum and 45°C for an additional 10 min, for a complete removal of organic solvent as indicated by visual observations. Vesicles were prepared by hydrating the lipid film in the presence of 10 ml phosphate buffer (pH 7.4). Liposomes formed were ultrasonicated for 40 mins to reduce the size of the vesicles and kept for overnight in order to mature the liposomes [15].

2.3.1 In-process Checks during Formulation of Sorafenib Liposomes:

RPM: 65-70 rpm (Film formation), 50-55 rpm (Hydration)

Temperature: 40-45°C (Film formation), 65-70°C (Hydration)

Characterization of Liposomes

The particle size and zeta potential have been determined for each formulation. Particle size of the formulations has determined using a transmission electron microscopy technique. The shape and morphology of the liposome droplet have been determined by scanning electron microscope at an accelerating voltage of 15KV and photomicrographs of suitable magnification was obtained [16,17].

Drug Content

Drug content in liposomes were assayed by an UV spectrophotometric method. Liposomes (5 mg) were dissolved in the mixture of PBS (pH 7.4) and methanol (1:9 v/v ratio) by shaking the mixture manually for 2 min. 1 ml of the resultant solution was taken and diluted with methanol upto 10 ml and then absorbance was recorded at 265 nm using a spectrophotometer and the concentration was obtained by using the equation of standard calibration curve.

Drug Entrapment Efficiency

Entrapment efficiency of liposomes has been determined using the centrifugation method. Liposomal dispersion was subjected to centrifugation on a laboratory centrifuge (Dolphin instrument) at 13,500 rpm for a period of 90 min at 4°C. The clear supernatants and sediment are carefully separated and absorbance of both have been checked and noted at 265 nm on UV spectrophotometer. Amount of Sorafenib in supernatant and sediment gives a total amount of Sorafenib in whole dispersion [18].

The Entrapment Efficiency was calculated by using the formula:

$$\% \text{ Entrapment efficiency} = \frac{\text{Entrapped drug}}{\text{Total drug amount added}} \times 100$$

In Vitro Drug Release Study

The release studies have carried out in 250 ml beaker containing 100 ml phosphate buffer (pH 7.4). The beaker is assembled on a magnetic stirrer and the medium has equilibrated at 37 ± 5ºC. Dialysis membrane is taken with one end of the membrane sealed that containing liposomal drug dispersion; suspended in the medium of phosphate buffer. Aliquots were withdrawn (5 ml) at specific intervals, filtered and the apparatus have immediately replenished with the same quantity of fresh buffer medium. Samples withdrawn have been subjected for UV absorbance [19].

Stability Studies

Stability studies have performed to inspect the leakage of the drug from the liposome during storage. Liposomal suspensions of Sorafenib tosylate of optimized formulations were sealed in 20 ml glass vials and stored at refrigeration temperature (4ºC), room temperature (25±2°C / 60± and at accelerated stability study (40±2°C / 75±5% RH) for a period of 90 days. Samples have been withdrawn and subjected for the evaluation at a definite time intervals [20].

RESULTS AND DISCUSSIONS

Standard Calibration Curve of Sorafenib in UV Spectrophotometer

The UV absorbance's of Sorafenib tosylate standard solutions in the range of 2-12 µg/ml of drug in buffer pH 7.4 showed linearity at λmax 265 nm. The linearity graph plotted for absorbance (A) against concentration (C) with R^2 value 0.999 and with the slope equation y=0.011 × -0.005. The absorbance values and standard curve were in figure 2.

Drug-Excipient Compatibility Studies

By analyzing the FTIR study of pure drug and formulation it has been concluded that there is no interaction between drug and excipients.

Drug Content and pH

The drug content study of all six batches have been calculated by dispersing the liposomes in mixture of buffer & methanol solution and assay was made by UV spectrophotometer. From all these batches F5 batch showed highest drug content (97.26 %) of drug. In all six batches F5 batch gives optimum pH.

Drug Entrapment Efficiency

The entrapment efficiency of all six batches has been determined by centrifugation method. From all these batches F5 batch that showed highest entrapment (55.62 %) of drug. Hence, F5 batch was considered as an optimized batch as given in below table 5.

Measurements of Particle Size and Zeta Potential Analysis

The measurement of particle size of vesicles of liposomal formulation was performed by TEM analysis. The measurement of Zeta potential allows for a prediction about the storage stability of colloidal particles, as the particle aggregation will be less to the charged particles. For the prepared liposomes the Zeta potential (mV) & particle size were tabulated in Table 6.

Determination of Surface Morphology

The shape and morphology of the liposome droplet determined by SEM showed that they are spherical, smooth vesicles of nanosize. The SEM image was shown in Figure 4.

In Vitro Drug Release Study

The in-vitro release of sorafenib tosylate liposomal formulations checked using the dialysis membrane and measured the release at 1 hr, 2 hr and 3 hr upto 24 hrs. After, examining percentage cumulative drug release graph of all formulation, the F5 batch showed a highest drug release (83.36 %) at 24hrs. Hence, the F5 batch was concluded as an optimized batch.

Stability Studies

Stability studies on optimized F5 batch of Sorafenib tosylate liposome preparation have been conducted for 90 days in different temperature conditions and have evaluated for physical appearance, pH, drug entrapment efficiency, drug content and in-vitro drug release as a function of the storage condition. The liposomes stored at 4ºC were found to be stable for the duration of 90 days as compared to room temperature and accelerated stability temperature. The results were showed in table 8.

CONCLUSIONS

The research conducted has showed the better suitability of poor bioavailable drug sorafenib tosylate towards the liposome encapsulation. Result has showed maximum drug release within 24 hrs (83.36 %) with good entrapment efficiency (55.62%) which can be achievable with liposome encapsulation. The work on the formulation development of Sorafenib tosylate liposomes was very much advantageous than the existing dosage forms as the drug is targeting to the cancerous cells, hence, it is a better action. The future studies are in-vitro cytotoxicity and in-vivo anticancer studies and estimation of targeting capacity of the liposomes in the cancer treatment for human.

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Figure and Table Legends

Figure 1 Structure of liposome

Figure 2 Standard calibration curve of Sorafenib drug

Figure 3(a) FTIR of Sorafenib drug

Figure 3(b) FTIR of Sorafenib tosylate liposomes

Figure 4 SEM analysis of optimized batch F5

Figure 5 Cumulative % drug releases of Sorafenib tosylate liposomes

Figure 6 Stability studies plot for optimized formulation F5qa

Figure 7 *In vitro* drug releases at initial and after stability

Table 1 Formulation of liposomes

Table 2 Standard calibration curve of drug by UV spectrophotometer in PBS 7.4

Table 3 Interpretations of FTIR spectra of liposomal formulation

Table 4 Drug content and pH

Table 5 Percentage of drug entrapment efficiency of liposomal formulation

Table 6 Particle size and Zeta potential analysis

Table 7 Invitro drug release of Sorafenib tosylate liposomes

Table 8 Stability studies of optimized batch

Table 9 Invitro release study of optimized formulation at initial and after stability study

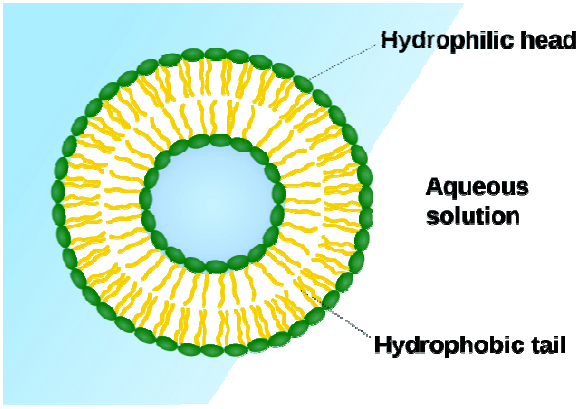

Figure 1: Structure of Liposome.

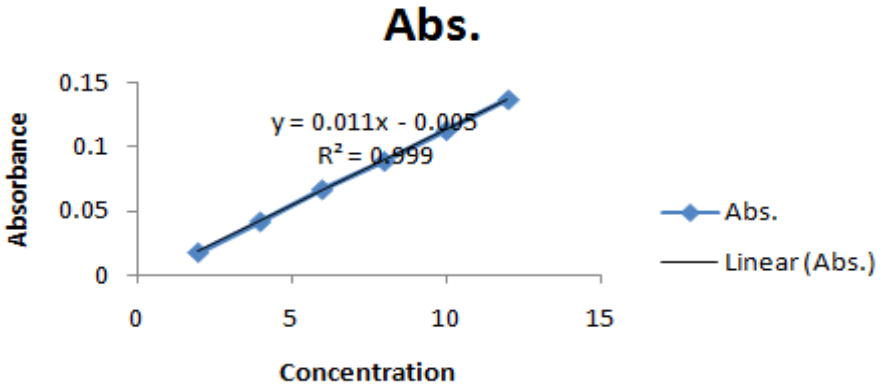

Figure 2: Standard Calibration Curve of Sorafenib Drug.

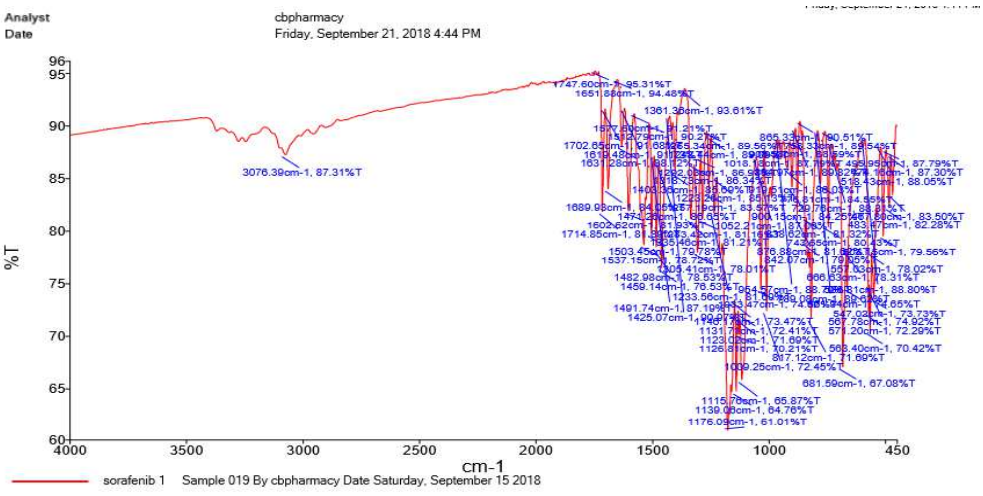

Figure 3(a): FTIR of Sorafenib Drug.

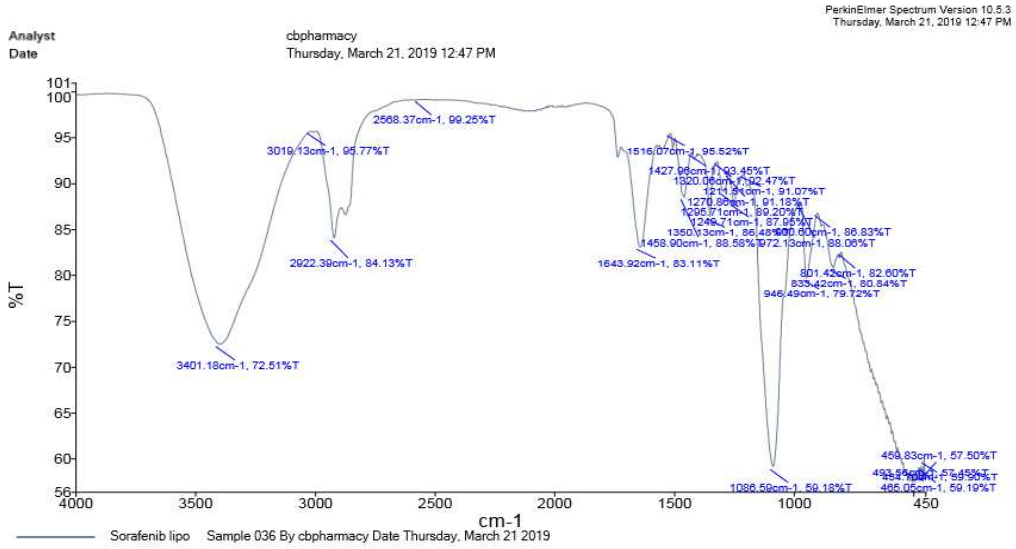

Figure 3(b): FTIR of Sorafenib Tosylate Liposomes.

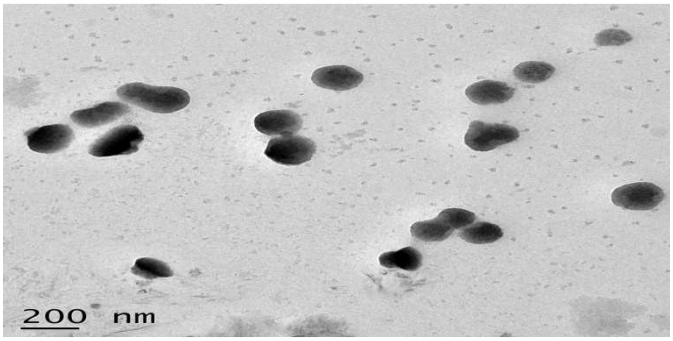

Figure 4: SEM Analysis of Optimized Batch F5.

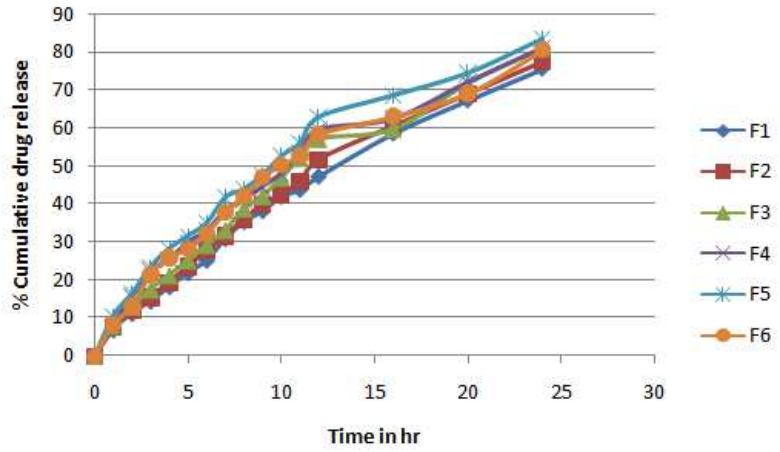

Figure 5: Cumulative % Drug Releases of Sorafenib Tosylate Liposomes.

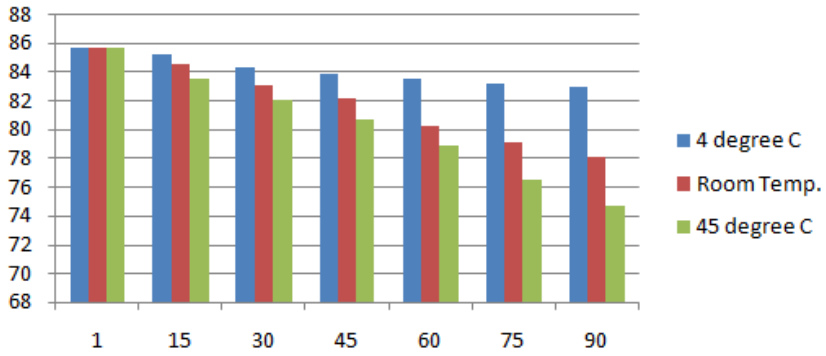

Figure 6: Stability Studies Plot for Optimized Formulation F5.

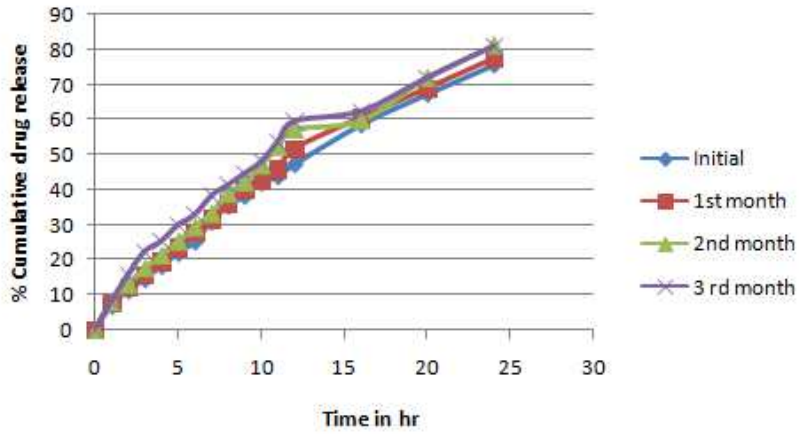

Figure 7: *In Vitro* Drug Release at Initial and After Stability.

Table 1: Formulation of Liposomes

Formulation code	Ingredient (mg/ml)						
	Drug (mg/ml)	HSPC (mg)	Cholesterol (mg)	Tween 80 (ml)	Chloroform (ml)	Methanol (ml)	PBS pH 7.4 (ml)
F1	10	40	13.33	0.25	3.5	1.5	10
F2	10	50	16.55	0.25	3.5	1.5	10
F3	10	60	20	0.25	3.5	1.5	10
F4	10	70	23.33	0.25	3.5	1.5	10
F5	10	80	26.66	0.25	3.5	1.5	10
F6	10	90	30	0.25	3.5	1.5	10

Table 2: Standard Calibration Curve of Drug by UV Spectrophotometer in PBS 7.4

Sr. No.	Concentration (µg/ml)	Absorbance at 265 nm
1.	2	0.018
2.	4	0.042
3.	6	0.067
4.	8	0.089
5.	10	0.113
6.	12	0.137

Table 3 Interpretations of FTIR Spectra of Liposomal Formulation

Sr. No.	Functional Groups	Standard Frequency	Observed Peak of Drug	Observed Peak of Formulation
1.	**C-H Stretching**	3040-3010	3076	3019
3.	**C=C Stretching**	1620-1680	1651	1643
4.	**N-H Bending**	1500-1650	1619	1516
5.	**C-O Stretching**	1250-1350	1361	1295

Table 4: Drug Content and pH

Sr. No.	Formulation Code	Drug Content (%)	pH
1.	F1	95.81	7.35
2.	F2	96.06	7.36
3.	F3	95.53	7.29
4.	F4	96.89	7.32
5.	F5	97.26	7.34
6.	F6	96.83	7.36

Table 5: Percentage of Drug Entrapment Efficiency of Liposomal Formulation

Sr. No.	Formulation Code	Percentage of Drug Entrapment Efficiency
1.	F1	79.86 %
2.	F2	81.56 %
3.	F3	82.91 %
4.	F4	83.79 %
5.	F5	85.62 %
6.	F6	82.76 %

Table 6: Particle Size and Zeta Potential Analysis

Formulation code	Size in nm	Zeta potential (mV)
F1	156.94	15.11
F2	145.83	15.2
F3	148.36	17.5
F4	135.84	15.21
F5	118.19	20.9
F6	152.43	19.6

Table 7: In Vitro Drug Release of Sorafenib Tosylate Liposomes

Time (hr)	% Cumulative drug release					
	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
1	6.89	7.5	7.88	8.16	10.48	8.26
2	11.22	12.03	12.93	15.76	16.38	13.04
3	14.44	15.48	17.56	22.36	23.40	21.43
4	18.22	19.42	21.25	25.30	28.20	25.95
5	21.88	23.52	25.22	29.90	31.52	28.04
6	25.15	27.81	29.26	32.80	34.91	32.22
7	30.86	31.59	33.11	38.19	41.65	37.68
8	35.36	35.95	38.84	41.29	43.81	42.04
9	38.16	39.83	42.09	44.58	47.55	46.98
10	41.93	42.5	46.90	48.01	52.54	50.25
11	43.73	45.81	52.12	53.72	55.90	52.68
12	47.19	51.62	57.13	59.52	62.75	58.34
16	58.52	60.43	59.70	62.34	68.47	62.95
20	67.25	68.88	71.83	72.01	74.37	69.05
24	75.56	77.34	81.09	80.97	83.36	80.43

Table 8: Stability Studies of Optimized Batch

Sr. No.	Number of days	Physical Appearance	pH	% Drug Entrapment efficiency			Drug Content (%)
				4^{o} C	Room Temp.	At 45^{0} C	
1	Initial	Milky white dispersion	7.34	85.62	85.62	85.62	97.26
2	15	No change	7.34	85.15	84.45	83.45	97.18
3	30	No change	7.35	84.23	83.06	81.96	97.08
4	45	No change	7.35	83.78	82.16	80.62	96.74
5	60	No change	7.30	83.46	80.23	78.83	96.12
6	75	No change	7.32	83.12	79.12	76.48	95.87
7	90	No change	7.20	82.96	78.09	74.68	95.11

Table 9: In Vitro Release Study of Optimized Formulation at Initial and After Stability Study

Time in hr	% Cumulative drug release after stability			
	Initial	At 4^{0} C	At Room Temp.	At 40^{0} C
0	0	0	0	0
1	10.48	9.84	9.34	8.56
2	16.38	15.39	14.18	14.23
3	23.40	22.18	21.36	20.53
4	28.20	27.48	25.13	24.16
5	31.52	30.58	28.12	28.65
6	34.91	35.03	32.45	31.48
7	41.65	40.75	38.12	35.26
8	43.81	42.93	42.85	40.12
9	47.55	46.59	46.12	44.23
10	52.54	52.23	51.18	48.65
11	55.90	54.06	54.81	52.96
12	62.75	60.97	59.26	58.61
16	68.47	66.45	64.78	62.86
20	74.37	72.68	69.13	67.91
24	83.36	82.74	80.58	77.13